PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference HY3APCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/FI2004/000346	International filing date *(day/month/year)* 07.06.2004	Priority date *(day/month/year)* 06.06.2003

International Patent Classification (IPC) or national classification and IPC
C12N15/10, C12N15/11, C12N7/00

Applicant
RNA-LINE OY et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.04.2005	18.08.2005

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2004/000346

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-40 as originally filed

Sequence listings part of the description, Pages

1, 2 as originally filed

Claims, Numbers

1-29 received on 12.04.2005 with letter of 06.04.2005

Drawings, Sheets

1/4-4/4 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 30,31
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-29
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Prior art

Reference is made to the following documents:

D1: WO 03/027330 A (3 April 2003)

D2: EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 19, (4 January 2000), pages 124-133 [XP002302296]

Item V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT)

V.1.1. None of the available prior art documents discloses a method according to claim 1 or a system or kit according to claims 25 and 29. The claims are therefore novel in the sense of Art. 33(29 PCT.

V.1.2. Document D1, which is considered as the closest proir art, discloses a method for protein evolution by using a RNA dependent RNA polymerase (RdRP) capable of shuffling between two homologous templates (see the relevant passages as defined in the ISR). The authors also contemplate an in vivo method where the RdRP is expressed in a cell together with the target nucleic acid and one screens for mutated proteins having the desirable characteristics.
Although, it was known from the prior art that the RdRP of bacteriophage $\phi 6$ is capable of replicating unspecifically heterologous RNA templates and that, as all of that class of viral replicases, it is devoid of proper proof-reading (see D2), it was nevertheless not obvious for the skilled person to combine the teachings of documents D1 and D2 to arrive at the subject-matter of the present claims which involve therefore an inventive step as defined by Art. 33(3) PCT.

V.2. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

The subject-matter of the present claims is considered as being industrially applicable in the sense of Art. 33(4) PCT.

Item VIII. Certain observations on the international application

Attention is drawn to present claims 25,26,28 and 29 which include human (embryos) in their scope. This subject-matter is considered by the EPO as being contrary to morality (Art. 53 EPC) and corresponding objections will be raised against said claims when entering into the regional phase before the EPO.

What is claimed is:

(96)

1. A method for changing a target nucleic acid sequence, the method comprising:

a) providing nucleic acid target in a form that can be replicated by a polymerase devoid of the proof-reading function;

b) incorporating the nucleic acid target into the genome of an RNA virus or other RNA replicon where said nucleic acid target is replicated by the polymerase encoded by the RNA virus or other RNA replicon under conditions sufficient for template-directed nucleic acid synthesis in a living cell; and

c) recovering nucleic acid synthesis products, whose nucleotide sequence differs from the initial target sequence by at least one nucleotide.

2. The method according to claim 1, wherein said nucleic acid target encodes a polypeptide.

3. The method according to claim 1 or 2, wherein said polymerase is an RNA-dependent RNA polymerase.

4. The method according to any one of claims 1 to 3, wherein said polymerase is an RNA-dependent DNA polymerase.

5. The method according to any one of the preceding claims, wherein the nucleic acid synthesis products are recovered after selecting and/or screening nucleic acid synthesis products based on their properties.

6. The method according to any one of the preceding claims, wherein said nucleic acid synthesis products are recovered after one or several rounds of selection and/or screening.

7. The method according to any one of the preceding claims, wherein the method is specifically used for changing properties of proteins or nucleic acids in a desired manner.

8. The method according to any one of the preceding claims, wherein the polymerase is a genetically modified or wild-type polymerase.

9. The method according to any one of the preceding claims, wherein the RNA virus or other RNA replicon is genetically modified or wild-type.

10. The method according to any one of the preceding claims, wherein the nucleic acid target is operably linked with determinants essential for detectable replication by the polymerase.

11. The method according to any one of the preceding claims, wherein the RNA replicon is an RNA virus-like particle, viroid or RNA-based autonomous genetic element.

12. The method according to any one of the preceding claims, wherein the nucleic acid encoding the polymerase and the target nucleic acid are distinct nucleic acids.

13. The method according to any one of the preceding claims, wherein the nucleic acid target is a nucleic acid having detectable biological activity, preferably selected from the group comprising enzymatic, regulatory and specific binding activity.

14. The method according to any one of the preceding claims, wherein the nucleic acid target encodes a protein having detectable biological activity, preferably selected from the group comprising enzymatic, regulatory and specific binding activity.

15. The method according to any one of the preceding claims, wherein the nucleic acid target is RNA.

16. The method according to any one of the preceding claims, wherein the nucleic acid target is DNA.

17. The method according to any one of the preceding claims, wherein the nucleic acid synthesis products are RNA molecules.

18. The method according any one of the preceding claims, wherein the nucleic acid synthesis products are DNA molecules.

19. The method according any one of the preceding claims, wherein the RNA virus is an RNA bacteriophage.

20.The method according to claim 19, wherein the RNA virus is from a member of the *Cystoviridae* family, preferably from a bacteriophage selected from the group comprising ϕ6, ϕ7, ϕ8, ϕ9, ϕ10, ϕ11, ϕ12, ϕ13 and ϕ14, most preferably from bacteriophage ϕ6.

21.The method according to any one of the preceding claims, wherein the replicable form of the nucleic acid target is replicated in a prokaryotic cell, preferably in a gram-negative bacterial cell, more preferably in a bacterial cell selected from the group comprising *Pseudomonas sp.*, *Escherichia sp.* and *Salmonella sp.*, most preferably in a cell of *Pseudomonas syringae*.

22. The method according to any one of claims 1 to 21, wherein the replicable form of the nucleic acid target is replicated in a eukaryotic cell, such as mammalian, insect, plant or yeast cell.

23. The method according to any one of the preceding claims, wherein the nucleic acid target is delivered into the living cell by using a suicide vector, preferably a DNA vector, most preferably a DNA plasmid.

24. The method according to any one of the preceding claims, wherein a suicide vector, comprising a target nucleic acid operably linked with sequences sufficient for detectable replication by the viral replication apparatus, is used to incorporate said nucleic acid target into the genome of said RNA virus.

25. A system for changing a target nucleic acid sequence, which comprises

- a target nucleic acid sequence operably linked with determinants essential for replication by an RNA synthesis apparatus of an RNA virus or another RNA replicon;

- a living cell capable of supporting the replication of the RNA virus or other RNA replicon; and
- a selection/screening procedure for selecting/screening a change in the properties of the nucleic acid synthesis products.

26. The system according to claim 25, wherein the RNA-synthesis apparatus is from a member of *Cystoviridae* family.

27. The system according to claim 25 or 26, wherein the living cells are bacteria, preferably gram-negative bacteria, more preferably bacteria selected from the group comprising *Pseudomonas sp.*, *Escherichia sp.* and *Salmonella sp.*, most preferably *Pseudomonas syringae*.

28. The system according to any one of claims 25 to 27, wherein the cells are carrier-state cells or can be transformed into carrier state.

29. A kit for changing nucleic acid or protein sequences, which comprises:
a) a vector for transient expression of target nucleic acid in preselected cells that either are carrier-state or can be transformed into carrier state and/or
b) a genetically modified virus into where the target nucleic acid can be introduced; and/or
c) cells that either are carrier-state or can be transformed into carrier state.

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